E. 9 Entrapping of *E. coli* in alginate beads

16.09.2014

Inoculation of over night cultures of JM 109 C2 Lac-end.

17.09.2014

Measurement of cell number: $5{,}8 \cdot 10^{10}$ using a Hemocytometer.

Washed in 0,9% saline solution

1 mL of that washed cultures was added to 200 ml of Saline solution. → plating efficiency test (1:10 – 1:1000000) to check how many cells survived the preparation so fare.

2 g alginate into 200 mL water, mixed for a long span of time, add 20 mL of diluted saline culture.

0,05 M $CaCl_2$ solution was prepared and the alginate culture solution was added → alginate beads formed.

Inoculation of:

Beads in 3 mL 2x YT

Beads in 3 mL rumen liquid

Over night (24h)

18.09.2014

Centrifugation of beads in rumen liquid and 2x YT for 5 min at 800 rpm. Supernatants were transferred into new falcons and centrifuged with E. coli in 2x YT and rumen liquid for 10 min at 4000 rpm.

Beads were washed in 0,9% NaCl and incubated in 3 mL 1x PBS for 1h at 37 °C and 250 rpm.

Supernatants were decanted and pellets were washed two times with 3 mL 1x PBS. Pellets were resuspended in 3 mL 1x PBS and then plated on Agar-Plates (Ca & Amp, Dilution 1:1000 & 1:10.000).

Beads were resuspended in 3 mL 1x PBS and plated on Agar-Plates (Ca & Amp, Dilution 1:100 & 1:10.000).

Incubation of plates over night at 37°C.

Results:

19.09.2014

Niels Rüdiger

The experiment from 18.09.2014 is repeated.

20.09.2014

Niels Rüdiger

The experiment from 18.09.2014 is repeated again.

→ Result: